

RESERVE CARBOHYDRATES IN SOUTH AFRICAN GRASSES.

By HANS WEINMANN AND LEONORA REINHOLD.

(Department of Botany, University of the Witwatersrand, Johannesburg.)

(WITH PLATE III.)

Figures in parentheses, see References at end of this article.

The significance of carbohydrate reserves in certain South African grasses has been discussed in various previous papers (12-15). The results of sugar and starch determinations led to the conclusion that these materials are the more important types of reserve carbohydrates in the species investigated. In recent years considerable attention has been focussed on the occurrence of fructosans in grasses and other monocotyledons (1, 4). Sullivan and Sprague (11) point out that fructosans may often have been reported as starch and dextrin, and consider that fructosan, rather than starch, is important as a reserve carbohydrate in grasses. As a contribution to the ultimate settlement of these controversies, the writers have now carried out detailed carbohydrate analyses on the roots, rhizomes and shoots of a number of important indigenous South African grasses, involving the determination of reducing and non-reducing sugars, fructosan, dextrin and starch in these materials. Carbohydrate analyses of various exotic grasses were included.

MATERIAL.

Roots and Rhizomes.—The species from which root and rhizome samples were taken are listed in Table I, together with data regarding localities and times of sampling. Wherever possible, the samples were taken from protected plots, and during autumn or winter, so as to ensure a high content of reserves. A number of plants of each species were dug up to a depth of at least four to six inches. The bulk of the adhering soil was removed by washing or shaking, and as soon as possible the material was taken to the laboratory, where it was killed by autoclaving at 5 lb. pressure for five minutes. The samples were then dried at a temperature of 50° to 60° C., cut up, ground and stored in air-tight bottles for chemical analysis.

TABLE I.

Roots and Rhizome Materials Investigated.

Species.	Sampling Date.	Locality.
<i>Brachiaria serrata</i>	8th August, 1942	Natural veld; plots protected from grazing for four seasons; old herbage removed by cutting every year in mid-winter.*
<i>Elyonurus argenteus</i>	19th June, 1942	
<i>Eragrostis chalcantha</i>	7th August, 1942	
<i>Harpechloa falx</i>	8th August, 1942	
<i>Microchloa caffra</i>	19th June, 1942	
<i>Monocymbium cerasiiforme</i>	21st June, 1942	
<i>Trachypogon plumosus</i>	16th June, 1942	
<i>Tristachya hispida</i>	18th June, 1942	
<i>Digitaria tricholaenoides</i> ..	10th August, 1942	Moderately grazed plots.*
<i>Cynodon dactylon</i> , Friel's Selection	13th July, 1943	Turf plot, protected for two years.* Intensively grazed <i>Cynodon</i> pasture.*
do., Wild Kweek grass	25th Sept., 1944	
<i>Hyparrhenia hirta</i>	6th July, 1945	Protected plot, burnt every alternate year in winter since 1931.†
<i>Themeda triandra</i>	6th July, 1945	
<i>Agrostis tenuis</i>	21st March, 1945	6 to 10-year-old nursery plots.*
<i>Arrhenatherum elatius</i>	19th March, 1945	
<i>Lolium perenne</i>	19th March, 1945	
<i>Phalaris arundinacea</i>	21st March, 1945	
<i>Chloris gayana</i>	10th July, 1945	Plot protected for two seasons.*
<i>Paspalum dilatatum</i>	10th July, 1945	
<i>Pennisetum clandestinum</i> ..	10th July, 1945	

* Botanical Research Station, Frankenwald, near Johannesburg.

† Crescent Creek Experimental Plots, Johannesburg.

Shoots.—During the season 1943-44 herbage samples of four species of grasses were collected in one of the experimental plots of the Witwatersrand University at Crescent Creek, Johannesburg. This plot had been burned every alternate year in June since 1931, but remained protected from any other interference. Cook (3), examining the plot in 1937, found that its vegetation had not changed appreciably since the first burn.

For the present investigation *Brachiaria serrata*, *Hyparrhenia hirta*, *Trachypogon plumosus* and *Themeda triandra* were chosen as test materials.

Herbage samples of these species were collected on 24th November, 1943, 15th February, 1944, 18th April, 1944, and 28th June, 1944. The last burn to which the plot was subjected before these samples were taken took place on 30th June, 1943. Information concerning the growth stage of the individual species on the sampling dates is given in Table II.

TABLE II.

Phenological Observations.

Sampling Date.	24th Nov., 1943.	15th Feb., 1944.	18th April, 1944.	28th June, 1944.
<i>Brachiaria serrata</i>	Leaves and stems; inflorescences formed	End of flowering period; inflorescences lost	All stems and most leaves dead	All aerial parts dead.
<i>Hyparrhenia hirta</i>	Leaves only formed	Flowering stage	Seeds being formed; yellowing	Do.
<i>Themeda triandra</i>	Inflorescences formed	End of flowering period; inflorescences still present	Leaves partly brown	Do.
<i>Trachypogon plumosus</i>	Leaves; stems being formed	End of flowering period; inflorescences lost	Leaves partly brown	Do.

The samples were always taken in the morning between nine and ten o'clock, and were killed immediately by autoclaving. The matériel was then dried and ground in the same way as the root and rhizome materials.

ANALYTICAL METHODS.

Sugars.—Sugars were extracted from 2.5-gram samples of the air-dry material by heating with 100 ml. of 95 per cent. alcohol for two hours. Some calcium carbonate was added to prevent acid hydrolysis during extraction. The alcohol was removed from the filtrate by distillation under reduced pressure, the remaining syrup taken up with water, cleared with neutral lead acetate, made to 100 ml. volume, and de-leaded with potassium oxalate. A 25-ml. aliquot was hydrolyzed by heating for half

an hour on a boiling water bath with 1.25 ml. of 25 per cent. hydrochloric acid. The reducing power of the sugar extracts was determined by a previously described semi-micro method (16). The latter was also used to estimate the reducing power of the other carbohydrate extracts prepared in this investigation.

Fructosans and Dextrins.—The residue from the sugar extraction was dried at 50 to 60° C., and transferred to a 500-ml. Erlenmeyer flask. 100 ml. of distilled water and a few drops of toluene were then added, and the mixture was agitated for two hours on a shaking apparatus. The liquid was filtered under suction through a dry filter on a Buchner funnel, without washing: 50 ml. of the aqueous extract were pipetted into a 250-ml. Erlenmeyer flask, and to these were added 20 ml. thymol-saturated acetic acid-sodium acetate buffer solution, pH 4.45, and 5 ml. of dialyzed takadiastase solution (corresponding to 25 mg. of original takadiastase, Parke, Davis & Co., Detroit, U.S.A.). The flasks were then tightly stoppered and incubated for 44 hours at 37 to 38° C. for the digestion of dextrins. At the end of the digestion period 1.1 ml. of 25 per cent. hydrochloric acid were run into the digests, and the flasks heated for half an hour on a covered bath of boiling water to hydrolyze fructosans (the liquid in the flasks reaching a temperature of approximately 75° C.). The hydrolysates were cooled down, three-quarters of the acid was neutralised with a pre-determined quantity of strong sodium hydroxide solution, the liquid was transferred to a 100-ml. flask, cleared with neutral lead acetate and made to volume. The filtered liquid was then neutralized with solid sodium carbonate to a pH of 5.0 to 5.5, the pH being checked by a Beckman pH meter, after which the solution was de-leaded with solid potassium oxalate.

For the determination of fructosans a modification of the colorimetric method developed by Roe (10) and Hubbard and Loomis (6) for blood and urine analyses was used. The method is based on the fact that fructose when heated with a solution of resorcinol in the presence of concentrated hydrochloric acid gives a red colour, the intensity of which, within certain limits, is proportional to the fructose concentration. One ml. of a 1 per cent. solution of resorcinol in 95 per cent. alcohol was added to 1 ml. of the cleared and de-leaded filtrate in a graduated test-tube, followed by 3 ml. of 30 per cent. hydrochloric acid. After being shaken to ensure adequate mixing, the tubes were heated for exactly eight minutes in a water-bath maintained at 80° C., and then rapidly cooled in running water. The resulting coloured solutions were made up to the volume of 5 ml. with 95 per cent. alcohol, after which they were compared in a Dubosque colorimeter with a standard fructose solution which had been treated simultaneously with the filtrates by

the same procedure. The method was tested on pure fructose solutions over a range of 0.02 to 0.4 mg. per ml., and found to give results accurate to well within 5 per cent. of the expected value. Concentrations below 0.02 mg. per ml. gave a colour that was still detectable, but not measurable in the Dubosque colorimeter. Superior sensitivity would probably be achieved with a photo-electric colorimeter.

Interference due to the presence of glucose was investigated in a series of experiments. It was found that concentrations lower than 1 mg. glucose per ml. did not produce a measurable colour. The fructose equivalent of higher concentrations of glucose (concentrations up to 50 mg. per ml. were tested) tended to vary slightly. The average figure for the apparent fructose value of 1 mg. glucose was 0.006 mg. Wherever discrepancies between total sugar estimations and fructose determinations indicate that more than 1 mg. glucose is present per ml., therefore, corrections for glucose should be made. These were unnecessary in the present investigation as the glucose concentration never approached 1 mg. per ml.

In order to estimate the possible interference by chromogens other than reducing sugars, McRary and Slattery (8) in a recent paper recommend fermentation of the hydrolyzed extracts previous to treatment with resorcinol. In the present investigation several extracts were subjected to rapid fermentation with baker's yeast (17). The fact that colourless solutions were subsequently obtained on heating with resorcinol indicated, firstly, that all the chromogens present in the solutions were fermentable, and, secondly, that no complex polysaccharides capable of being hydrolyzed to chromogens under the strongly acid conditions obtaining for colour development were present.

It should be noted that some extracts were observed to yield deep-red solutions when heated with concentrated hydrochloric acid even in the absence of resorcinol. The presence of alcohol, however, inhibited this colour formation, which thus did not interfere with the estimations.

From the results of the determinations of fructose and of the total reducing power, the fructosan and dextrin content of the samples was calculated, taking into account the weight increase of these substances upon hydrolysis as well as the lower reducing power of fructose as compared with that of glucose (16).

Starch.—The residue from the water extraction was thoroughly washed, and, following the usual gelatinization treatment, was digested with 3 ml. saliva for 20 hours at 37 to 38° C.; 50 ml. of the cleared and de-leaded digest were hydrolyzed with 4 ml. of 25 per cent. hydrochloric acid in an autoclave at 15 lb. pressure for one hour. Starch was calculated by multiplying the glucose value by the factor 0.9.

Ash and Dry Matter.—Dry matter determinations were carried out on all samples, and in roots and rhizomes the ash content was determined in addition. The analytical results were reported as percentages of the dry matter in the case of the shoots, and as percentages of the ash-free (combustible) dry matter in the case of the roots and rhizomes. Errors due to variations in the amounts of sand and soil adhering to the underground parts were thus eliminated.

DISCUSSION OF RESULTS.

I. RESERVE CARBOHYDRATES IN UNDERGROUND PARTS.

The results of the carbohydrate determinations in roots and rhizomes are given in Table III. As will be seen from these figures, reducing sugars, occurred only in small amounts in most materials. Exceptions were the roots of *Elyonurus argenteus* (1·04 per cent.) and *Chloris gayana* (2·24 per cent.), and the roots and rhizomes of *Pennisetum clandestinum* (4·95 and 2·81 per cent. respectively). Non-reducing sugars, on the other hand, were found in appreciable amounts in the majority of the materials investigated. In 12 out of the 14 root samples of indigenous South African species, non-reducing sugars occurred in larger amounts than did the other types of carbohydrates. Amongst the exotic species this was the case only in *Paspalum dilatatum* and *Pennisetum clandestinum*, both species being indigenous to tropical countries. While considerable quantities of non-reducing sugars were also recorded in most of the rhizomes, it was only in those of *Digitaria tricholaenoides* that these compounds were present in excess over the other reserve carbohydrates.

No significant amounts of fructosans were found in the roots and rhizomes of any of the indigenous South African grasses, the highest concentration being reached in *Chloris gayana* with 0·70 per cent. Fructosans were, however, abundant in the roots of all the exotic grasses investigated. In the roots of four of the exotic species studied fructosans constituted the principal carbohydrate. These species were *Agrostis tenuis*, *Arrhenatherum elatius*, *Lolium perenne* and *Phalaris arundinacea*, all indigenous to cool temperate climates. The occurrence of fructosans in these, or closely allied, species has been reported by previous workers (1, 4, 11). Particularly large amounts of fructosans were found in the rhizomes of *Agrostis tenuis* and *Phalaris arundinacea* (viz., 17 and 31 per cent. respectively).

Dextrins were generally found to be either absent or occurring only in very small quantities. It may be not without significance that the presence of somewhat larger amounts of dextrins was usually associated with a high starch content (roots of *Cynodon dactylon*, wild Kweek grass,

TABLE III.

Reserve Carbohydrates in the Underground Parts of Grasses.

Expressed as percentages of the combustible dry matter.*

Species.	Red. Sugars.	Non-red. Sugars.	Fructo- sans.	Dex- trins.	Starch.	Total.
<i>Indigenous South African Grasses.</i>						
Roots.						
<i>Brachiaria serrata</i> ..	0.15	6.30	0.00	0.00	0.13	6.58
<i>Chloris gayana</i> ..	2.24	4.69	0.70	0.09	0.03	7.75
<i>Cynodon dactylon</i> :						
Friel's Selection ..	0.06	1.65	0.00	0.06	1.99	3.76
Wild Kweek Grass † ..	0.28	6.05	0.34	0.42	5.86	12.95
<i>Digitaria trichol.</i> ..	0.48	6.20	0.26	0.18	0.18	7.30
<i>Elyonurus argenteus</i> ..	1.04	6.90	0.34	0.34	0.28	8.90
<i>Eragrostis calcantha</i> ..	0.08	2.68	0.00	0.60	0.13	2.89
<i>Harpechloa falx</i> ..	0.19	2.50	0.20	0.28	1.15	4.32
<i>Hyparrhenia hirta</i> ..	0.85	10.50	0.57	0.00	0.29	12.21
<i>Microchloa caffra</i> ..	0.03	1.90	0.00	0.00	0.13	2.06
<i>Monocymbium ceres.</i> ..	0.21	4.51	0.00	0.00	0.14	4.86
<i>Themeda triandra</i> ..	0.19	8.75	0.41	0.00	0.04	9.39
<i>Trachypogon plumos.</i> ..	0.08	3.76	0.00	0.00	0.14	3.98
<i>Tristachya hispida</i> ..	0.01	1.26	0.32	0.44	7.73	9.76
Rhizomes.						
<i>Cynodon dactylon</i> :						
Friel's Selection ..	0.12	2.50	0.55	0.87	12.53	16.57
Wild Kweek Grass † ..	0.23	3.90	0.20	0.72	10.28	15.33
<i>Digitaria trichol.</i> ..	0.26	0.95	0.00	0.28	0.41	1.90
<i>Exotic Grasses.</i>						
Roots.						
<i>Agrostis tenuis</i> ..	0.14	0.43	2.61	0.00	0.48	3.66
<i>Arrhenatherum elatius</i> ..	0.23	0.85	1.92	0.36	0.67	4.03
<i>Lolium perenne</i> ..	0.03	0.33	3.03	0.19	0.25	3.83
<i>Paspalum dilatatum</i> ..	0.46	11.38	1.24	0.05	0.15	13.28
<i>Pennisetum clandest.</i> ..	4.95	7.41	1.13	0.23	0.19	13.91
<i>Phalaris arundinacea</i> ..	0.22	1.61	3.44	0.00	0.24	5.51
Rhizomes.						
<i>Agrostis tenuis.</i> ..	0.61	0.64	16.75	0.14	0.95	19.09
<i>Pennisetum clandest.</i> ..	2.81	3.89	0.69	1.19	4.78	13.36
<i>Phalaris arundinacea</i> ..	0.78	3.16	31.15	0.54	0.63	36.26

* The most abundant carbohydrate group in each species is given in bold type.

† Analysed by Miss E. Goldsmith. Starch determined by digestion with *α*-amylase.

and of *Tristachya hispida*; rhizomes of *Cynodon dactylon* and of *Pennisetum clandestinum*). The dextrin content exceeded 1 per cent. only in the rhizomes of *Pennisetum clandestinum*.

Though the analytical methods used, involving digestion with saliva, indicated the presence of starch in the roots and rhizomes of all species, only a few contained starch in large amounts and as the principal carbohydrate. The highest percentages of starch were recorded in the rhizomes of *Cynodon dactylon* (viz., 10.3 and 12.5 per cent.). A similarly high starch content, namely 13.4 per cent., has been recorded in *Cynodon dactylon* rhizomes by De Cugnac (4), whereas Julander, a recent American worker, considers that not starch but "glucosans" (i.e., dextrans) are the important form of carbohydrate reserve in this grass (7). Another instance of a high starch concentration is provided by the roots of *Tristachya hispida* (7.7 per cent.). This finding is in agreement with previous results obtained by the senior author (13, 15).

In addition to the chemical analyses, qualitative starch tests were carried out on the tissues of ten of the native species. Sections of leaves, stems, roots and rhizomes were cut, treated with iodine solution, and examined microscopically. The results are summarised in Table IV.

TABLE IV.

Results of Qualitative Starch Tests in Tissues of South African Grasses.

Species.	Leaves.	Stems.	Roots.	Rhizomes.
<i>Brachiaria serrata</i>	+ (1) (2)	—	—	
<i>Cynodon dactylon</i>	+ (1) (2)	+++ (3)	++ (6)	+++ (4)
<i>Digitaria tricholaenoides</i>	+ (1) (2)	++ (3)	+	++ (3)
<i>Elyonurus argenteus</i>	+ (1)	—	—	
<i>Eragrostis calcantha</i>	+ (1)	++ (3)	+	
<i>Harpechloa falx</i>	+ (1) (2)	—	++ (6)	
<i>Hyparrhenia hirta</i>	+ (1) (2)	—	—	
<i>Themeda triandra</i>	+ (2)	+	—	
<i>Trachypogon plumosus</i>	+ (1) (2)	—	—	
<i>Tristachya hispida</i>	+ (1) (2)	++ (3)	++ (6)	

KEY :

— = Starch absent.

+ = Starch present in small amounts.

++ = Starch present in moderate amounts.

+++ = Starch present in large amounts.

(1) Starch present in outer bundle sheath.

(2) Starch present in leaf sheath, at junction with node, between each fibro-vascular bundle and inner epidermis.

(3) Starch present in parenchyma of cortex and ground tissue.

(4) Starch present in parenchyma of ground tissue.

(5) Starch present in parenchyma of ground tissue at node only.

(6) Starch present in pith parenchyma.

Starch was detectable in all the species, not only in the subterranean organs, but also in the aerial parts. Where starch was present in the leaf blades, it invariably occurred in the chloroplasts of the outer bundle sheath. The colour obtained here with iodine solution was usually, however, not the typical blue, but rather a black-brown, even when the chlorophyll had been extracted with alcohol previous to testing. It is nevertheless considered that the stain was due to small amounts of starch. Typical blue starch grains were found in the leaf sheaths at the junction with the node, even in species where the iodine test gave doubtful or negative results in the leaf blades. The accumulation of starch in the plastids of the bundle sheath has been observed by Rhoades and Carvalho (9) in maize and sorghum.

The results of the qualitative starch tests on the roots and rhizomes largely confirmed those of the chemical analysis. In the three species where quantitative analysis revealed the presence of larger amounts of starch (viz., *Cynodon dactylon*, *Harpechloa fax* and *Tristachya hispida*) distinct accumulations of typically-stained starch grains were found in the tissues. Positive tests were also given by the roots of *Digitaria tricholaenoides* and *Eragrostis chalcantha*. The rhizomes of *Cynodon dactylon* were strikingly rich in starch (see Plate III).

That the results of the chemical analysis indicated very small amounts of starch in a number of species where no starch could be detected microscopically, may be due to the hydrolysis by saliva of substances other than starch (5) or else to incomplete extraction of sugars or fructosans, a small fraction of which may have remained in the residue used for the starch determination. This was most probably also the case in the roots and rhizomes of *Agrostis tenuis*, *Arrhenatherum elatius*, *Lolium perenne* and *Phalaris arundinacea*, where no starch grains could be detected, though chemical analysis indicated small quantities of starch.

According to De Cugnac (4), grasses can be divided into two groups, namely, those which accumulate in their vegetative organs sucrose with or without starch ("Graminées saccharifères"), and those which store fructosan, usually together with sucrose ("Graminées lévulifères"). De Cugnac's tentative suggestion that the latter group essentially comprises grasses native to cool temperate climates, while the "Graminées saccharifères" are mostly adapted to warm regions, seems to be confirmed by the results of the present investigation. In none of the indigenous South African grasses studied did fructosans occur in appreciable amounts. Most of the grasses contained non-reducing sugars, presumably in the form of sucrose, as their principal carbohydrate. Some contained high percentages of starch, the latter carbohydrate actually constituting the main carbohydrate reserve in several cases. General statements offered

by some workers asserting the negligible importance of starch as a reserve carbohydrate in the vegetative organs of grasses are thus shown to be not permissible, since the type of carbohydrate reserve is apparently largely a characteristic of the species concerned.

II. SEASONAL CARBOHYDRATE TRENDS IN THE SHOOTS OF FOUR SOUTH AFRICAN HIGHVELD GRASSES.

The results of the herbage analyses are indicated in Table V and Figure 1. The shoots of all four species examined contained reducing

TABLE V.

Seasonal Carbohydrate Trends in the Shoots of Four South African Highveld Grasses.

Expressed as percentage of the dry matter.

Sampling Date.	Reducing Sugars.	Non-reducing Sugars.	Total Sugars.	Starch.	Total available Carbohydrate.*
<i>Brachiaria serrata.</i>					
24/11/1943	0.42	1.23	1.65	0.69	2.34
15/ 2/1944	0.43	0.99	1.42	0.30	1.72
18/ 4/1944	1.29	0.75	2.04	0.23	2.27
28/ 6/1944	0.39	0.09	0.48	0.22	0.70
<i>Hyparrhenia hirta.</i>					
24/11/1943	0.68	1.68	2.36	0.70	3.06
15/ 2/1944	0.59	1.20	1.79	0.09	1.88
18/ 4/1944	1.64	1.98	3.62	0.04	3.66
28/ 6/1944	2.26	0.32	2.58	0.06	2.64
<i>Themeda triandra.</i>					
24/11/1943	1.26	2.22	3.48	0.64	4.12
15/ 2/1944	0.45	2.08	2.53	0.19	2.72
18/ 4/1944	1.67	1.98	3.65	0.07	3.72
28/ 6/1944	1.05	0.25	1.30	0.13	1.43
<i>Trachypogon plumosus.</i>					
24/11/1943	0.66	1.37	2.03	0.42	2.45
15/ 2/1944	0.17	0.98	1.15	0.26	1.41
18/ 4/1944	0.74	0.97	1.71	0.13	1.84
28/ 6/1944	0.63	0.29	0.92	0.31	1.23

* Total available carbohydrate = combined sugars and starch.

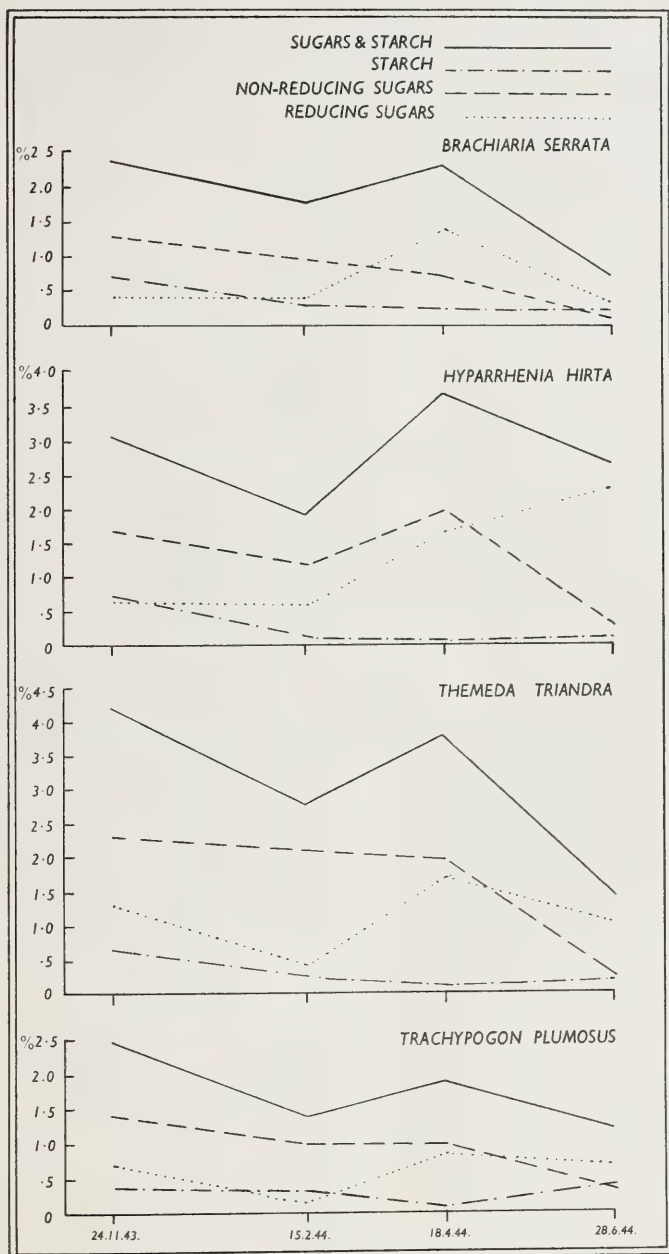


FIG. 1. Seasonal carbohydrate trends in the shoots of four South African Highveld grasses.

sugars, non-reducing sugars and starch, whereas fructosans and dextrans could not be detected in measurable quantities. There was much variation in the relative proportions of these substances, but in all instances sugars were present in larger quantities than starch. Total sugars ranged from 0.48 to 3.65 per cent., and starch from 0.04 to 0.70 per cent.

Total sugars decreased in the four species in early summer (i.e., between 24th November and 15th February) and rose again in late summer and early autumn. In three of the four species the maximum percentage of total sugars was recorded on 18th April, 1944. Starch, on the other hand, occurred in maximum amounts in all species in spring (24th November), and decreased distinctly with the advancing season, though in three species somewhat higher amounts were found in winter (28th June) than in autumn (18th April). Total sugars, as well as the combined percentages of sugars and starch, showed a distinct fall during late autumn and winter (i.e., from 18th April to 28th June).

It is known that the sugar and starch content of leaves is subject to considerable diurnal variations, largely depending on meteorological conditions influencing the rates of photosynthesis and respiration. Carbohydrates elaborated in the leaves are used to build up new tissues or are temporarily stored in the stems. While meteorological conditions prevailing on the sampling dates may have influenced the results to some extent, it is considered that the recorded changes in carbohydrates largely represent seasonal variations, since entire shoots were analysed, and not only the leaves. As considerable growth took place in all four species after the first sampling date, involving the formation of stems and inflorescences, the decrease in the percentages of sugars and starch between the first and second sampling date does not necessarily imply a decrease in amount. On the contrary, it is highly probable that these substances increased in actual amount between 24th November and 15th February. The decrease in the percentages of sugars and starch during this period is probably due to the fact that dry matter increased more rapidly in amount than did these carbohydrates. By 15th February the flowering period was over in three of the species, while *Hyparrhenia hirta* was still in the flowering stage. The flowering period marks the end of rapid expansion of the aerial parts, and is followed by the period of maturation. It is significant that during the latter stage (i.e., between the second and third sampling date) total sugars as well as combined sugars and starch increased appreciably in percentage in all four species. When conditions are favourable for photosynthesis, and no further growth is taking place, carbohydrates are elaborated in excess after flowering, and consequently increase in percentage.

When the third sample was taken (18th April) typical autumn

weather had already set in, though no frosts had occurred. The process of ageing and gradual death of the aerial portions during late autumn was associated with the loss of an appreciable portion of the accumulated sugar. Some of this sugar may have been utilised in the aerial parts in respiration or lost by decomposition, while part of it was probably translocated to the roots. As shown in earlier investigations (13), the sugar content in the roots of Highveld grasses usually increases from February onwards, i.e., soon after flowering. At this stage the excess of carbohydrates elaborated by the plant is so great that these compounds increase in percentage (and actual amount) in the aerial parts though appreciable portions are translocated to the roots at the same time. The removal of carbohydrates from shoots to roots is, however, not a complete one, as considerable amounts of sugars and starch were found in the herbage collected in winter (28th June). These ranged from approximately 30 to 70 per cent. of the amounts present in autumn (18th April). Bunting (2), who determined the sugar content of the stem bases of three grass species harvested at Frankenwald between 18th July and 6th August, 1937, obtained the following values:—

Total Sugars as Percentage of Combustible Dry Matter.

Species.	Range.	Average.
<i>Tristachya hispida</i>	0.89 — 9.95	2.91
<i>Trachypogon plumosus</i>	1.28 — 7.30	3.11
<i>Brachiaria serrata</i>	1.62 — 5.01	2.48

It may be assumed that the carbohydrates which are temporarily stored in the stem bases can be utilised for new growth, at least as long as these plant parts are alive. Such utilisation may occur, for instance, after mowing. Whether the residue of sugars and starch in old stems can be utilised for spring growth in the following season, is, however, not known.

Table VI and Figures 2 and 3 show the relative proportions of reducing sugars and starch. Reducing sugars, expressed as percentage of total sugars, increased in the shoots of *Brachiaria serrata* and *Hyparrhenia hirta* right through the season, and in *Themeda triandra* and *Trachypogon plumosus* at least from February onwards. Thus with the advancing season reducing sugars increased at the expense of non-reducing sugars, so that in winter the former exceeded the latter in actual amount (Fig. 1 and Table V). These changes can be interpreted as being due to increased

TABLE VI.

Relative Proportions of Reducing Sugars and Starch in the Shoots of Four South African Highveld Grasses.

Sampling Date.	<i>Brachiaria serrata.</i>	<i>Hyparrhenia hirta.</i>	<i>Themeda triandra.</i>	<i>Trachypogon plumosus.</i>
Reducing Sugars.				
Expressed as percentage of total sugars.				
24/11/1943	25.5	28.9	36.2	32.5
15/ 2/1944	30.3	33.0	17.8	14.8
18/ 4/1944	63.2	45.3	45.7	43.3
28/ 6/1944	81.3	87.6	80.8	68.3
Starch.				
Expressed as percentage of total available carbohydrate.*				
24/11/1943	29.5	22.9	15.5	17.2
15/ 2/1944	17.5	4.8	7.0	18.5
18/ 4/1944	10.1	1.1	1.9	7.1
28/ 6/1944	31.4	2.3	9.1	25.2

* Total available carbohydrate = combined sugars and starch.

hydrolysis of sucrose, and to a more rapid rate of translocation of sucrose, as compared with that of hexoses.

Starch, expressed as percentage of total available carbohydrate,* decreased in three of the four species between 24th November and 18th April, and in *Trachypogon plumosus* between 15th February and 18th April. This indicates that starch synthesis was most active during the early stages of growth and gave way to hydrolysis of starch with ageing and maturation. In winter, however, there was an increase in the relative proportion of starch. Why the winter starch content of the dry matter in three of the four species exceeded that in autumn (Table V and Fig. 1), is difficult to explain. Though it is possible that during late autumn and early winter some starch was formed from sugars or by the break-down of more complex polysaccharides, it is more likely that these fluctuations were due to sampling or other errors, particularly since the percentages of starch were in most instances very small.

* "Total available carbohydrate", in this case, means combined amounts of sugars and starch, since fructosans and dextrins were absent.

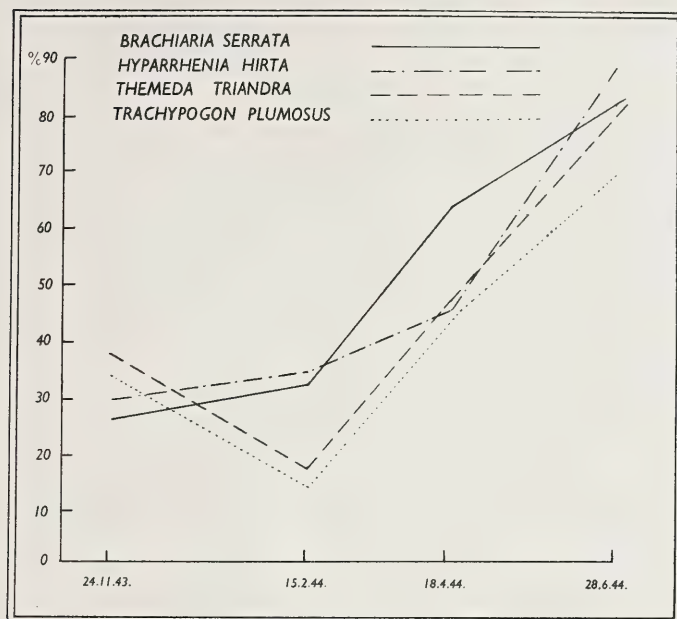


FIG. 2. Reducing sugars in the shoots of four South African Highveld grasses (expressed as percentage of total sugars).

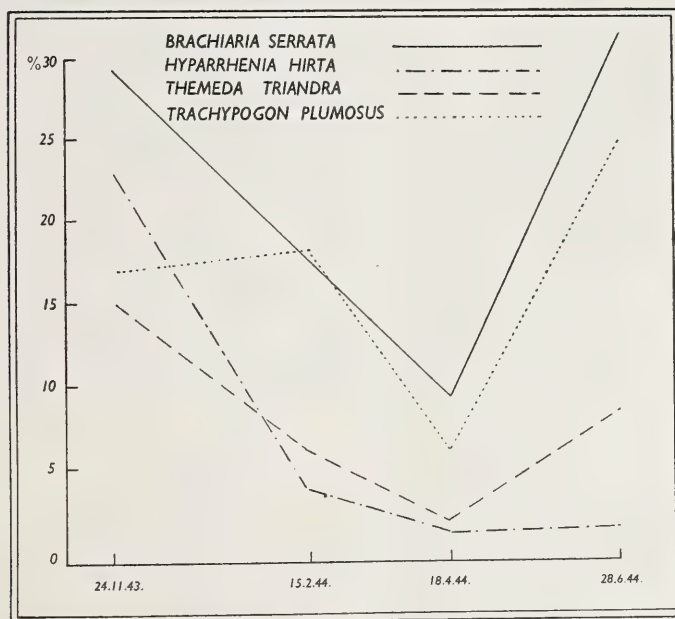


FIG. 3. Starch in the shoots of four South African Highveld grasses (expressed as percentage of total available carbohydrate).

SUMMARY.

The results of carbohydrate determinations on the roots and rhizomes of thirteen indigenous South African grasses and of six exotic grasses are reported and discussed. Reducing and non-reducing sugars, fructosans, dextrans and starch were estimated in this material.

In the majority of the South African species investigated, non-reducing sugars formed the principal reserve carbohydrate, while in some starch occurred in largest amounts. Results of qualitative tests for starch in sections confirmed the presence of this carbohydrate in both aerial and underground parts of a number of species.

None of the South African species studied was found to contain appreciable amounts of fructosans or dextrans. The presence of considerable quantities of fructosans was confirmed in four of the exotic grasses in which fructosans had previously been reported.

Seasonal carbohydrate trends were investigated in the shoots of four indigenous South African grasses. The shoots of all four species were found to contain reducing sugars, non-reducing sugars and starch, but no fructosans or dextrans; sugars were present in larger amounts than starch.

Total sugars, as well as combined sugars and starch, increased in the shoots of these grasses after flowering, and decreased during maturation, i.e., in autumn and early winter. The results suggested that carbohydrates are elaborated in the leaves in excess after flowering, and are consequently translocated to the roots, though considerable portions of sugars and starch remain in the aerial parts during winter.

A new technique of estimating fructosans and dextrans is described.

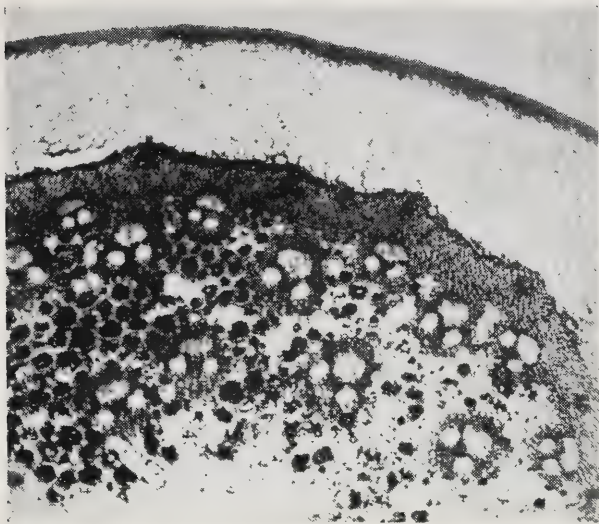
ACKNOWLEDGMENTS.

The writers wish to express their sincere thanks to Professor John Phillips, Head of the Department of Botany, University of the Witwatersrand, for having provided the facilities which made this research possible.

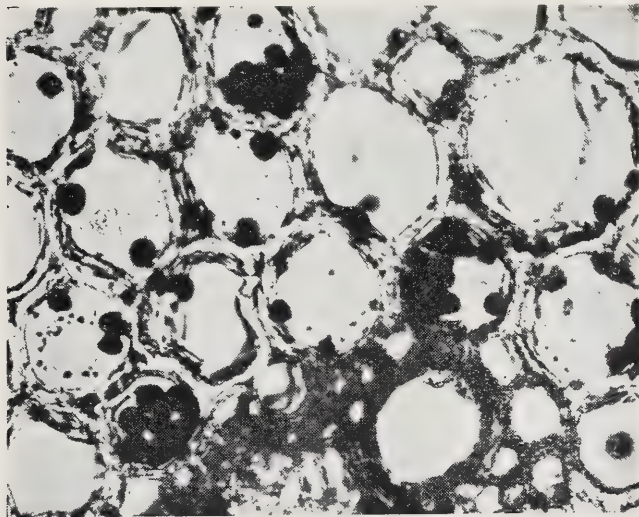
Thanks are also due to Miss E. Goldsmith and Miss N. Katz for their co-operation, and to Mr. A. Salbany for the photographs.

REFERENCES.

- (1) ARCHBOLD, H. K.: "Fructosans in the Monocotyledons." A review. *The New Phytologist* 39: 185-219, 1940.
- (2) BUNTING, A.: "The Effect of Combined Clipping and Chemical Treatments on the Root Reserves and Development of some Highveld Grasses." Unpublished M.Sc. Thesis, University of the Witwatersrand, 1938.
- (3) COOK, L.: "A Report on the Veld-burning Experiments at Crescent Creek, Milner Park, Johannesburg." Unpublished B.Sc. Hons. Thesis, University of the Witwatersrand, 1938.
- (4) DE CUGNAC, A.: "Recherches sur les Glucides des Graminées." *Annales des Sciences Naturelles*, 10e séries, 13: 1-129, 1931.
- (5) DENNY, F. E.: "Improvements in Methods of Determining Starch in Plant Tissues." *Contributions from Boyce Thompson Institute*, 6: 129-146, 1934.
- (6) HUBBARD, R. S., and LOOMIS, T. A.: "The Determination of Inulin." *Jour. Biol. Chem.* 145: 641-645, 1942.
- (7) JULANDER, O.: "Drought Resistance in Range and Pasture Grasses." *Plant Physiology* 20: 573-599, 1945.
- (8) MCRARY, W. L., and SLATTERY, M. C.: "The Colorimetric Determination of Fructosan in Plant Material." *Jour. Biol. Chem.* 157: 161-167, 1945.
- (9) RHOADES, M. M., and CARVALHO, A.: "The Function and Structure of the Parenchyma Sheath Plastids of the Maize Leaf." *Bull. Torrey Bot. Club* 71: 335-346, 1944. (Ref. Biol. Abstr. 18, No. 20461, 1944.)
- (10) ROE, J. H.: "A Colorimetric Method for the Determination of Fructose in Blood and Urine." *Journ. Biol. Chem.* 107: 15-22, 1934.
- (11) SULLIVAN, J. T., and SPRAGUE, V. G.: "Composition of the Roots and Stubble of Perennial Ryegrass following Partial Defoliation." *Plant Physiology* 18: 656-670, 1943.
- (12) WEINMANN, H.: "Storage of Root Reserves in Rhodes Grass." *Plant Physiology* 15: 467-484, 1940.
- (13) ——— "Seasonal Chemical Changes in the Roots of some South African Highveld Grasses." *Jour. S.A. Bot.* 6: 131-145, 1940.
- (14) ——— "Effects of Defoliation Intensity and Fertilizer Treatment on Transvaal Highveld. *Emp. Jour. Exp. Agr.* 11: 113-124, 1943.
- (15) ——— "Root Reserves of South African Highveld Grasses in Relation to Fertilizing and Frequency of Clipping." *Jour. S.A. Bot.* 10: 37-54, 1944.
- (16) ——— "Semi-micro Estimation of Reducing Sugars." *Plant Physiology* 19: 148-156, 1944.
- (17) YEMM, E. W.: "The Respiration of Barley Plants: I. Methods for the Determination of Carbohydrates in Leaves." *Proc. Roy. Soc. London, Series B*, 117: 483-504, 1935.



Micro-photograph of transverse section of rhizome of *Cynodon dactylon*, stained with iodine solution (magnification $92\times$). The section was so cut as to decrease in thickness from left to right. The intact parenchymatous cells of the ground tissue on the left are densely packed with starch grains, in the right-hand part many starch grains have fallen out so that individual starch grains can be seen in the cells.



Micro-photograph of transverse section of rhizome of *Cynodon dactylon*, stained with iodine solution (magnification $510\times$). Showing individual starch grains in parenchymatous cells of the ground tissue.